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Association of Polymorphism in Leptin Gene with Semen Quality Parameters in Egyptian Buffaloes Bulls

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Abstract

THIS study aimed to identify the single nucleotide polymorphisms (SNPs) in a partial sequence of the leptin gene and its potential association with the semen quality parameters and mRNA expression levels of the leptin gene in the Egyptian buffalo' bulls. A total of 20 Egyptian buffalo bulls were used in this study. The selected amplicon of the leptin gene was amplified using a specific primer. After that, the SNPs detection and genotyping were performed based on sequence analysis and high-resolution melting (HRM)-PCR. A c.216A>G synonymous SNP (g.14328A>G) was identified in the assessed partial leptin sequence. This SNP showed significant linkages with the semen profile. In this regard, the heterozygous AG genotype exhibited significantly higher progressive motility (PR), sperm concentration, total sperm count, and higher LH and testosterone hormone levels. While the GG genotype showed higher normal morphology form % and functional sperm %. Moreover, AG and GG genotypes displayed significant upregulation of leptin mRNA levels compared to the AA genotype. The g.14328A>G SNP could be considered an effective marker in marker-assisted selection and breeding programs for improving the fertility of Egyptian buffalo bulls.

Keywords: *Leptin*, Egyptian buffalo**,** SNP, HRM-PCR, fertility**.**

Introduction

Proper selection of superior breeding males is crucial for the effectiveness and success of a selective breeding program. Hence, males impact the transmission of good traits from parents to their descendants as one semen ejaculate could artificially inseminate thousands of females [1, 2]. Male fertility is affected by several factors such as mating ability, sex drive, and semen quality [3]. However, dissimilar to females, male fertility has received less attention in selective breeding programs. Selecting a superior bull is based mainly on its reproductive characteristics including semen characteristics such as sperm concentration, and motility, and the proportion of abnormal sperms along with the conception rate [4]. However, most of these traits have low to moderate heritability percentages [5] which strengthens the incorporation of genetic selection techniques to facilitate male selection [6].

There are several candidate genes modulating the semen characteristics [7]. The leptin (LEP) gene is one of these effective genes that regulate male

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reproductive performance via the hypothalamic-pituitary-gonadal (HPG) axis [8]. It controls the growth of testicular germ cells and the differentiation of spermatocytes to spermatids through the STAT3 signaling pathway [9]. Moreover, LEP plays a role in the survival and capacitation of sperm [10]. The leptin-deficient mice incorporated atrophied testes with hollow seminiferous tubules, decreased spermatogenesis, increased death of germ cells, and elevated expression of pro-apoptotic genes inside the testes [11]. Leptin is an autocrine and paracrine stimulant in the tissues of several species [12]. Elevation of the concentration of leptin hormone positively correlated with early puberty in male lambs will improve breeding value [13]. A few previous studies have shown the association of leptin gene polymorphism with buffalo bull reproductive features [14]. Therefore, the current study was done on Egyptian buffalo bulls to identify the proposed polymorphisms in the leptin sequence and its association with semen quality characteristics and the concentration of male hormones (LH and testosterone). They were besides, exploring the association of the detected SNPs with the mRNA expression levels of the leptin gene in Egyptian buffalo's spermatozoa.

Material and Methods

Buffalos bull source and management

The research proposal of this study was approved by the Institutional Animal Care and Use Committee (IACUC), Kafrelsheikh University, Egypt. (Approval number KFS-IACUC/114/2023). Twenty pure Egyptian water buffalo (*Bubalus bubalis*) males (bulls) were involved in this study. The animals were obtained from a local farm in Kafrelshiekh governorate, a Bulls-growing up farm, Mahalet Mousa, animal production research station, a branch of the Animal Production Research Institute, Agriculture Research Centre. The animals used in this study were exposed to similar housing and dietary systems. Plus, they weighed 450 ±50 kg live body weight. They were fed on Egyptian Berseem in the winter season while in the summer, they received a concentrated feed containing 16% crude protein, rice straw, and maize silage. Feed was usually offered twice daily (at 7 a.m. and 3 p.m).

Blood samples (n=20) were collected into vacuum tubes with an anticoagulant (disodium EDTA) and then kept at (-20oC) till DNA extraction and another sample was collected into plain tubes for hormonal analysis. From the same bulls, fresh semen ejaculates were collected, half of the sample was maintained at 37.5oC for semen analysis, and the second half was rapidly stored in liquid nitrogen for RNA extraction.

Semen and Hormonal analysis

Hormonal assays for plasma testosterone and luteinizing (LH) levels were conducted using commercial sandwich ELISA kits following the manufacturer's instructions. Sperm profile parameters including ejaculate volume, sperm morphology, percent of abnormalities, and functional sperm concentration, were also, determined according to the methods of Kumar et al and Eldebaky et al [15, 16].

Genomic DNA extraction and conventional Polymerase chain reaction (PCR)

The DNA was isolated using a whole blood genomic DNA Extraction kit (GeneJET, Thermo Fisher Scientific), following the kit's procedures. The extracted DNA integrity was verified using 1.5 % agarose gel electrophoresis and examined with an ultraviolet transilluminator (SYNGGENE, India) [17]. A specific primer has been used for the amplification of a clone of the leptin gene (327 bp spanning exon 3) (Table 1). The primer was established by software (Primer3-web version 4.1.0) according to the accession number XM_044946369.2.

The PCR reaction was carried out using a 2x PCR master mix (Applied Biotechnology, Egypt). The reaction mixture was composed of 2 µl DNA template, 0.5µl from each primer, and 25 µl master mix. The thermo-cycling conditions were done according to the methods of El-Nahas et al. [17]. Then, 2% ethidium bromide agarose gel electrophoresis was used to detect the PCR products.

DNA Sequencing

The PCR products of the chosen samples (n=10) were purified with a PCR purification kit (GeneJET, Thermo Fisher Scientific). An automated sequencer (Solgent, Korea) was used to sequence the purified PCR products. The sequenced PCR product was analyzed according to the method of Abdo et al. [18].

High melting resolution real-time PCR (HRM-PCR) for animal genotyping

The HRM-PCR was used to detect the differences between the heterozygote and homozygote genotypes (n=10), depending on the variation in the melting temperature of each sample, compared to a normalized fluorescence curve (the curve of previously sequenced samples with a known genotype were served as a reference samples in the real-time PCR run to normalize the melting curve data) [19, 20]. Briefly, the amplification was performed using a Real-Time PCR System (PikoReal 24, Thermoscientific, TCR0024). A total mix of 20 µL total reaction volume, including 2 µL of total DNA, 0.5 µL of primers, and 10 µL of 2x qPCR SYBR mix (Applied Bio tech. Egypt). The thermocycling amplification was done following the traditional real-time PCR condition with an annealing step at 60 °C for 30 seconds.

RNA extraction and reverse transcription

Before RNA extraction, semen samples were defrosted on ice and then centrifuged at 500 xg (2400 rpm) for 10 min at 4oC to discard seminal plasma. Then, the sperm cell pellet was washed with 1ml PBS (1X) and centrifugation at 500xg for 7 min. After that, 500 µl lysis buffer was added to the spermatozoa pellet, to confirm the absence of somatic cells, mixed well by vortex, and incubated for 15 min according to Ibrahim et al. [21]. The RNA extraction was done using the TRI reagent (easy-RED™, iNtRON Biotechnology), according to the manufacturer's guidelines. The quality of the RNA was confirmed using gel electrophoresis through visual inspection of rRNA bands (18S and 28S) in 2% agarose stained with ethidium bromide. The extracted RNA was reverse transcribed to the cDNA using the SensiFAST™ cDNA synthesis kit (Bioline, United Kingdom).

Real-Time PCR

The relative mRNA transcription level of the leptin gene was measured using a specific primer (Table 1). A Real-time PCR (qPCR) was performed using a 2x qPCR SYBR mix (Applied Biotechnology. Egypt). The reaction mix was 25 µl, containing 1 µl cDNA and 0.5 µl for each primer. The amplification conditions were as follows: 30 s of initial denaturation at 95 °C, after those 40 cycles of denaturation at 95 °C for 10 s, and 30 seconds of annealing (annealing temperature shown in Table 1). Data were normalized against (β-actin) as a housekeeping gene and the wild genotype (AA). Fold changes were calculated according to the method described by Livak, and Schmittgen [22].

Statistical analysis

The statistical linkage between the identified SNPs and semen quality parameters was analyzed using the GLM procedures of SPSS version 22, based on the following model: Yit= µ+ Gi + eik. In this model, Yit donates to the assessed semen quality parameters, LH, and testosterone levels. The symbol µ denotes the overall population mean, Gi represents the effect of the reported genotypes based on the detected SNPs (homozygous or heterozygous), and eik accounts for the residual effect. The genotype and gene frequencies were estimated using PopGene32 software and the Hardy-Weinberg equilibrium (HWE) was employed using the chi-square test ($\chi 2$). The semen quality parameters LH, and testosterone levels were presented as means ± standard errors.

Genetic diversity indices were calculated using a specific online tool called GenCal (https://gene-calc.pl/) which is used to calculate the polymorphic information content (PIC). While, the minor allele frequency (MAF), observed and expected heterozygosity (Ho & He, respectively), and the effective number of alleles (Ne) were estimated by Haploview 4.2 (Cambridge, MA, USA).

One-way ANOVA was also, performed to test the statistical significance in the mRNA expression level of the leptin gene between the different genotypes using GraphPad Prism 9 (©GraphPrism Software, La Jolla, California, USA). Moreover, the Pearson correlation coefficient was calculated to conduct a linear correlation matrix between the semen quality parameters, LH, and testosterone levels, and the mRNA expression levels of the leptin gene.

Results

Leptin gene SNPs

The amplified PCR product of the Leptin gene spanning exon 3, showed a specific DNA band at the target size (327bp) (Fig 1 A). Alignment of the sequenced amplicon revealed a transition SNP A>G (c.216A>G; g.14328A>G, which is located at 14328bp of the Leptin gene) (Fig 1 B). The SNP is a synonymous SNP (as it is located at the third position of the amino acid code).

The HRM real-time PCR was used to detect the different animals' genotypes. The melting curves of studied samples were arranged in 3 clusters evidenced against a normalized curve fluorescence (Fig 2 A & B). The HRM results showed 3 genotypes at position 216 including the AA wild type (blue-colored line), the AG heterologous mutation (red-colored line), and the GG homologous mutation (green-colored line).

Leptin genotypes and genetic indices

The genotypes and allele frequencies within the amplified sequence of the Leptin gene are shown in (Table 2). Three genotypes were identified AA, GG, and AG with genotype frequencies 0.15, 0.70, and 0.15, respectively. The allele frequencies for A and G alleles were 0.225 and 0.775, respectively. The other genetic indices including, the observed Ho and expected heterozygosity (He), the effective number of alleles (Ne), polymorphic information content (PIC), and the minor allele frequency (MAF) were 0.15, 0.3542, 1.548, 0.291, and 0.225, respectively (Table 3).

The association of the detected Leptin SNP with the semen profile parameters and plasma hormone level

The different semen quality parameters and their associations with the different genotypes are listed in Table 4 displaying significant differences in the semen prolife between the different genotypes. In this context, the heterozygous genotype AG showed a significantly higher sperm concentration and total sperm count compared to the homozygous genotypes AA and GG ($P < 0.05$). For the sperm's progressive motility (PR), the fastest motility was significantly associated with the A allele compared to the G allele ($P < 0.05$). in this regard, similar PR was reported for

the AA and AG genotypes ($P > 0.05$) which was significantly greater than that of the GG genotype ($P < 0.05$). On the other hand, the functional sperm % was significantly lower in the AG genotype compared with the AA and AG genotypes ($P < 0.05$). The functional sperm concentration (FSC) was similar in both AA and AG genotypes, while the GG genotype showed a significantly higher % ($P < 0.05$). The analyzed Teratozospermic index (TZI), sperm deformity index (SDI), normal morphology form %, and semen vitality showed slight non-significant differences among the 3 genotypes ($P > 0.05$).

Regarding the plasma testosterone and LH hormones concentration, both hormones were significantly higher in the heterozygous genotype AG ($P < 0.05$), while their concentration was nearly the same in the homozygous genotypes AA and GG ($P > 0.05$).

Leptin mRNA expression in sperm cells and its correlation with semen parameters

The relative mRNA levels of the leptin gene in the AG and GG genotypes were significantly higher than the wild genotype AA ($P < 0.05$). Moreover, the homozygous genotype GG showed the highest expression level ($P < 0.05$) (Fig .3).

The correlation, of leptin mRNA with the assessed semen characteristics showed a positive correlation with SC, NMF, FS%, FSC, Vitality, and TS (0.64, 0.51, 0.46, 0.05, 0.88, 0.21, respectively). In contrast, negative correlations were found with LEP mRNA and PM, TSC, TZI, SDI, and LH (-0.77, -0.45, -0.48, 0.48, -0.41, respectively) (Table 5).

Blasting of the sequenced Egyptian buffalo Leptin amplicon against the GenBank database showed a high homology percent with the related buffalo populations, shown in (**Table 6**). The highest identity (99.64%) was found with the online Leptin sequences of Murrah, Mediterranean, and Jafarabadi buffalo. Whereas the lowest identity (95.44 %) was found with the online sequence of the Leptin gene in the Egyptian buffalo.

Discussion

The Leptin gene is an important gene associated with male fertility. It regulates male fertility through stimulating the GnRH, FSH, and LH secretion [23, 24]. Besides, it has been proven for its effectiveness in regulating spermatogenesis and sperm quality [10, 25]. Mutation in the Leptin gene could impact male fertility. Accordingly, the leptin-mutant mice exhibit infertility, low levels of gonadotropins, and delayed puberty [11, 26]. However, it is still unclear how the Leptin gene mutations are related to Egyptian buffalo bull fertility and the quality of their semen [27].

Previous studies have recorded the SNPs in the exons and the non-coding sequences of Egyptian buffalo Leptin and their association with animal performance [18, 28]. SNPs in the exon could change the amino acid codon to another one which is called a non-synonymous mutation which consequentially could modulate the corresponding gene expression and the animal performance [29, 30]. On the other side, when SNPs occur in the third position of the translation codon, it doesn't change the amino acid, and this is referred to as synonymous SNPs [2]. These synonymous SNPs have been proven for their strong impact and association with gene expression and animal production [2, 31]. In our result, we identified a synonymous SNP c.216 A>G (g.14328 A>G) in exon 3 of the Leptin gene, with three identified genotypes (AA, AG, and GG genotype). Although this SNP did not change the amino acid, it was associated with a variable semen profile quality and male plasma hormones (LH and testosterone) concentrations. These impacts of the recorded synonymous SNP may be correlated with its influence on the mRNA half-life or the ribosome occupancy time which is reflected in the translation efficiency and rate [32, 33].

The heterozygous AG genotype of the SNP c.216 A>G showed a significantly higher total sperm count, and increased LH and testosterone levels. The increased levels of LH might be correlated with the reported increases in the total sperm counts (TSC) which was confirmed by the strong positive correlation (0.61) between the LH and TSC. This effect is probably because the plasma gonadotropins LH and FSH are crucial in the connections between the gonads and the pituitary gland, and their deficiency may lead to a disturbance of testicular function and infertility [10, 23]. In the same line, Akhter et al [34] suggested the stimulatory role of Leptin on gonadotropic cells and FSH and LH secretion. So, the higher sperm concentration and total sperm count with the AG genotype could be related to higher LH and testosterone hormone levels.

Our results also, demonstrate that the homozygous genotypes, AA, and GG of c.216A>G SNP showed a higher normal morphology form % and functional sperm % than the AG genotype. while The AA and AG genotypes increase Progressive motility (PR). A similar study in sheep showed a correlation between leptin polymorphism and sperm motility and fertility [27], it reported that the AA genotype of 170G>A SNP in exon 3 of the leptin gene had a higher motility trait. These reported improved semen parameters in the case of AG and GG genotypes may be linked with the enhanced mRNA level of the Leptin gene in the spermatozoa of these genotypes. On the other side, the non-synonymous SNP, 523G>A in the buffalo bull leptin gene, which resulted in an amino acid shift from arginine to glutamine hurt sperm motility after freezing and does not affect ejaculatory volume,

sperm concentration, and live and dead sperm percentage [35].

Our results demonstrated also, an association between the SNP c.216 A>G in Leptin and the viability of sperms. Similarly, Ishikawa and his colleagues [36] suggested that leptin may have a role in the regulation of the spermatozoa's physiological function as it is expressed in the germ cells, mainly in the spermatocytes. So, the Leptin is vital to the maintenance of sperm viability. Where the in vitro addition of leptin could maintain the viability and motility of sperm in the cooled semen of buffaloes [37]. It is also important for the strength of the sperm cell wall [38]. Also, SNP at 332G>A influence on the viability, water test, and scrotal circumference, with the AG genotype exhibiting the highest levels of these features.

The association between Leptin SNPs and animal fertility has been studied in different animal species. Accordingly, in cattle, the reported g.92450765 G > A SNP was recognized in the leptin gene with a significant effect on fertility [39]. The AA genotype of this SNP has a lower value of service period (SP), calving interval (CI), and artificial insemination (AI) per conception resulting in decreased reproductive disorders and improved fertility. Also, the allele G of this SNP displayed an increase in the milk yield. Moreover, previous studies have demonstrated that non-synonymous polymorphism (c.357C>T; A59V) in exon 3 of the LEP gene, was markedly associated with fertility as cows served and calved two weeks earlier for the first time also increased leptin concentration during pregnancy [40, 41]. On the other hand, Jecminkova et al [42] reported that the TT genotype of Leptin c.357C>T increases days open and calving interval.

Conclusion

The identified SNPs in the Leptin gene (c.216A>G) showed significant associations with bull semen quality, the plasma hormones concentration, and the relative expression of this gene in the sperm cells. This study recommended leptin as a candidate gene associated with bull semen quality. Moreover, this study holds practical implications for buffalo breeders aiming to enhance the reproductive performance of their animals through selective breeding strategies. However, it is recommended to conduct extensive further research using larger sample sizes to prove these findings and assess their pertinency for marker-assisted selection.

TABLE 1. Sequence of forward and reverse primers

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)	Ta (°c)	Size (bp)	Accession No/ Reference
Primer used for the isolation of *Leptin* (conventional PCR)					
LEP	CAGTCCGTCTCCTCCAAACAGAG	CATGTCCTGTAGTGACCCCTGCAG	60	327bp spanning exon 3	XM_044946369.2
Primer used in real-time PCR					
β-Actin	ACCGCAAATGCTTCTAGG	ATCCAACCGACTGCTGTC	60	199	NM_001290932.1 [47]
LEP	TGCAGTCTGTCTCCTCCAAA	CGATAATTGGATCACATTTCTG	62	152	NM_001290901

Ta: Annealing Temperatures

bp: Size of PCR Products

TABLE 2. Gene and genotype frequencies

Gene	SNP	Genotype Frequency			Allele Frequency		Chi-square (χ^2)	p-value
		AA	GG	AG	A	G		
LEP	g.14328A>G	0.15	0.70	0.15	0.225	0.775	6.305	<0.001

TABLE 3. Genetic indices of the identified SNPs

Gene	SNP	Ho	He	Ne	PIC	MAF	Alleles
LEP	g.14328A>G	0.15	0.3542	1.548	0.291	0.225	G: A

Ho & He = observed and expected heterozygosity, respectively. Ne = the effective number of alleles. PIC = polymorphic information content, minor allele frequency (MAF).

TABLE 4. Association of g.14328A>G SNP in LEP gene with semen quality of buffaloes' bulls

	Genotypes of g.16855A>G SNP of *LEP*			
	AA	**AG**	**GG**	**P values**
Progressive motility (PR)	66.16 ± 2.88[a]	69.94 ± 4.60 [a]	38.41 ± 3.40 [b]	0.001
Sperm concentration	55.63 ± 7.50 [b]	99.41 ± 3.77 [a]	52.25 ±4.43 [b]	0.001
Total sperm count	142.59 ±11.15 [c]	323.27 ± 12.86 [a]	264.13 ± 21.38 [b]	< 0.001
Normal morphology form %	49.30 ±4.46 [a]	38.45 ±4.89 [b]	52.13 ± 1.79 [a]	0.118
Functional sperm %	40.40 ± 3.47 [a]	25.63 ± 4.09 [b]	40.43 ±3.78 [a]	0.036
Functional sperm concentration (FSC)	54.40 ± 6.77 [b]	64.20 ±7.20 [b]	108.83 ± 15.29 [a]	0.01
Teratozospermic index (TZI)	2.08 ± 0.19 [a]	2.65 ± 0.47 [a]	1.90 ± 0.08 [a]	0.288
Sperm deformity index (SDI)	1.08 ± 0.18 [a]	1.65 ± 0.46 [a]	0.90 ± 0.08 [a]	0.288
Vitality	87.41 ± 11.53 [a]	91.69 ± 4.68 [a]	90.95 ± 8.73 [a]	0.932
Testosterone	0.14 ± 0.009 [b]	0.19 ± 0.001 [a]	0.13 ± 0.001 [b]	< 0.001
LH	0.40 ± 0.018 [b]	0.88 ± 0.009 [a]	0.37 ± 0.006 [b]	0.001

Results expressed as Means ± SE

TABLE 5. Correlation coefficients of LEP mRNA and semen profile parameters

	LEP mRNA	**PM**	**SC**	**TSC**	**NMF**	**FS%**	**FSC**	**TZI**	**SDI**	**Vitality**	**TS**	**LH**
LEP mRNA		0.00	-0.10	0.85	0.54	0.65	0.04	0.76	0.03	-0.45	0.87	-0.50
PM	-0.77		0.95	-0.72	0.80	-0.11	0.30	-0.33	-0.37	-0.89	-0.51	-0.86
SC	0.64	-0.52		-0.54	0.52	-0.74	-0.99	-0.26	-0.56	-0.70	-0.76	-0.65
TSC	-0.45	0.32	-0.97		-0.18	0.31	0.03	0.74	0.01	0.03	1.00	-0.03
NMF	0.51	-0.10	0.91	-0.65		-0.03	-0.10	-0.39	-0.64	-0.99	0.11	-0.99
FS%	0.46	-0.06	0.74	-0.74	0.44		0.69	0.45	0.67	0.83	0.60	0.80
FSC	0.05	-0.46	-0.20	0.34	-0.09	-0.82		0.02	0.42	0.62	0.82	0.57
TZI	-0.48	0.06	-0.89	0.84	-0.92	-0.74	0.41		0.48	0.99	-0.11	0.99
SDI	-0.48	0.06	-0.89	0.84	-0.92	-0.74	0.41	1.00		0.99	-0.11	0.99
Vitality	0.88	-0.83	0.89	-0.75	0.82	0.49	0.15	-0.69	-0.69		0.06	1.00
TS	0.21	0.19	-0.06	0.24	0.22	-0.69	0.94	0.14	0.14	0.35		0.00
LH	-0.41	0.22	-0.97	0.61	-0.90	-0.13	-0.29	0.74	0.74	-0.96	0.49	

PM= progressive motility; SC= sperm concentration; TSC= total sperm count; NMF= normal morphology form; FS%= functional sperm %; FSC= Functional sperm concentration; TZI= Teratozospermic index; SDI= Sperm deformity index; TS= testosterone; LH= Luteinizing hormone. Up for the GG genotype while down for the AG genotype

TABLE 6. Leptin gene online blasting

Animal	**Max Score**	**Total Score**	**Query Cover**	**E-value**	**Identity %**	**Acc. Number**
Murrah	510	510	100 %	1e-141	99.64	VDCC01000008.1
Mediterranean	510	510	100 %	1e-141	99.64	PZYV01000023.1
Jafarabadi	510	510	100 %	1e-141	99.64	ACZF03023079.1
Bangladesh	505	505	100 %	5e-140	99.28	NPZD01230639.1
Bubalus carabanensis breed swamp buffalo	505	505	100 %	5e-140	99.28	JARFXY010000008.1
Bubalus depressicornis	505	505	100 %	5e-140	99.28	JAMXBS010002680.1
Egyptian buffalo	448	448	99%	9e-123	95.44	LPUW01091341.1

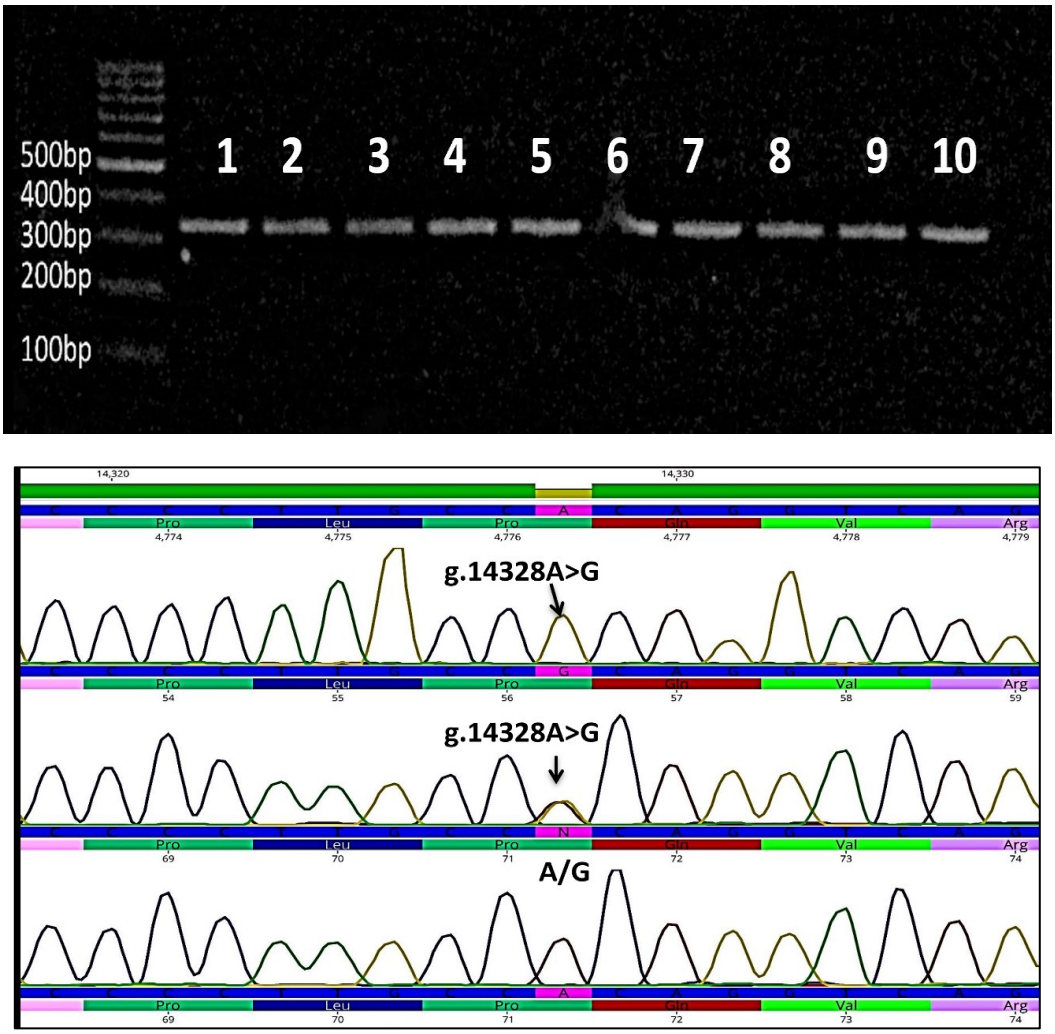

Fig. 1. (A) PCR result on an agarose gel stained with ethidium bromide of buffalo *Leptin* gene (exon 3); target size 327 bp. (B) The sequence of exon 3 of the buffalo *Leptin* indicates the synonymous SNP c.216A>G (g.14328A>G).

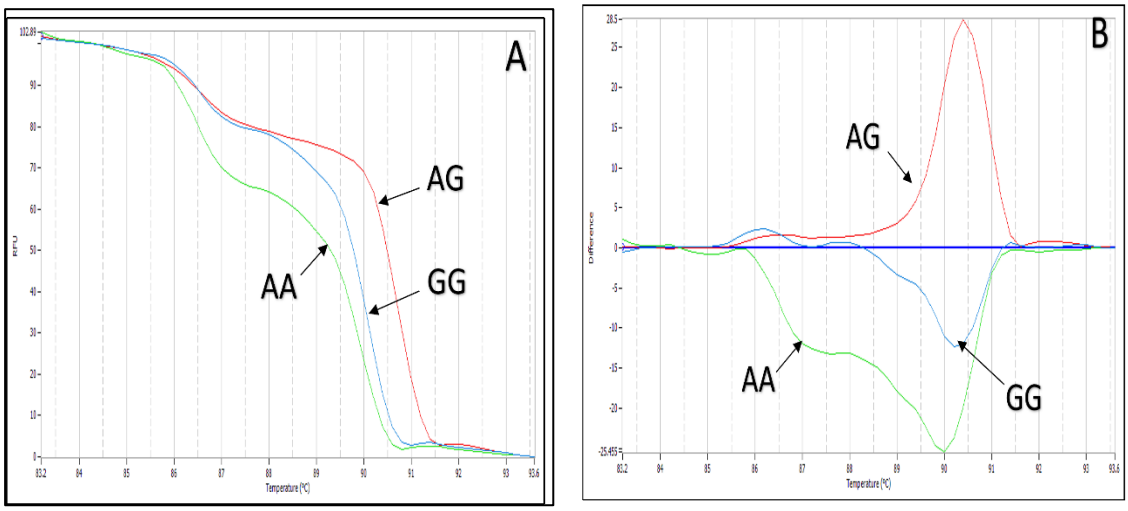

Fig. 2. PCR-HRM results in which the relative fluorescence unit is on the Y-axis and temperature in °C is on the X-axis. (A) The normalized melt curve of *the Leptin* gene (exon 3) shows 3 genotypes at c.216A>G SNP. (B) Difference plot curve of *Leptin* gene (exon 3) showing 3 genotypes at c.216A>G SNP.

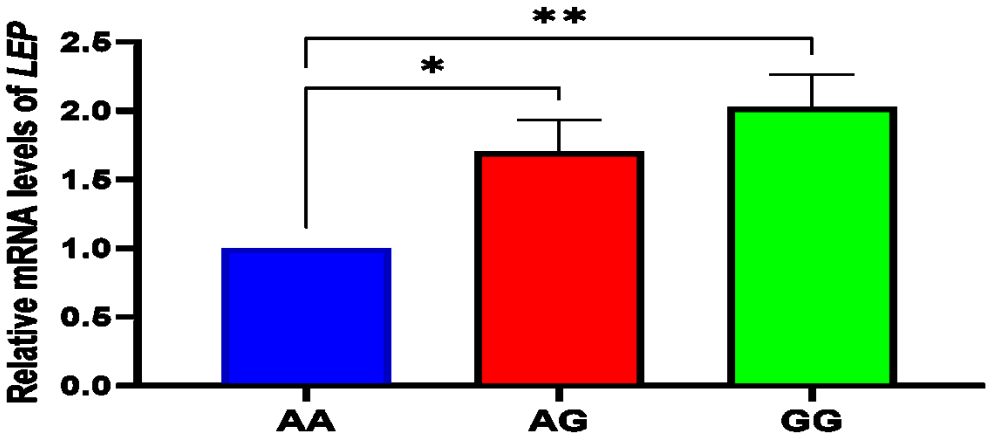

Fig. 3. Relative mRNA level of the Leptin gene of animals with different genotypes AA, AG, and GG (A). Data presented as a fold change. Data were normalized against β-actin as the housekeeping gene and the wild genotype AA.

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علاقة الطفرات في جين اللبتين مع مقاييس جودة السائل المنوي في ذكور الجاموس المصري

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الملخص

استهدفت هذه الدراسة التعرف على الطفرات الموضوعية (SNPs) في جزء من التسلسل الجيني لجين اللبتين ومدي ارتباطها بمقاييس جودة السائل المنوي ومستويات التعبير mRNA لجين اللبتين في ذكور الجاموس المصري. تم استخدام عدد 20 ذكر جاموس مصري في هذه الدراسة. تم عزل الجزء (الأمبليكون) المحدد لجين اللبتين باستخدام بريمر خاص. تم تحديد الطفرات والنمط الجيني من خلال تحليل التتابع النيوكليتيدي وتفاعل البلمرة المتسلسل .(HRM-PCR) تم التعرف على طفره موضوعية واحده، وهي c.216A>G SNP (g.14328A>G)في جين اللبتين. أظهرت هذه الطفرة ارتباط مهم مع تحليل السائل المنوي. في هذا الصدد، أظهر النمط الجيني AG الهجين لـ c.216A>G SNP اعلي حركية تقدمية (PR) ، وتركيز الحيوانات المنوية، وإجمالي عدد الحيوانات المنوية، ومستويات أعلى من هرمون LH وهرمون التستوستيرون. بينما أظهر النمط الجيني المتماثل GG أعلى شكل طبيعي و وظيفه للحيوانات المنوية. علاوة على ذلك، أظهرت الأنماط الجينية AG و GG زيادة ملحوظة في مستويات التعبير mRNA اللبتين مقارنة بالنمط الجيني .AA يمكن اعتبار الطفرة المكتشفة بمثابة علامة فعالة في برامج الاختيار والتربية بمساعدة الواسمات لتحسين خصوبة الجاموس المصري.

الكلمات الدالة: اللبتين، الجاموس المصري، الطفرات الموضوعية وتفاعل البلمرة المتسلسل (HRM-PCR)، خصوبة الذكر.